

### Occurrence of ketostearic acids in cow's milk fat: suggested intermediates in unsaturated fatty acid biosynthesis\*

Our study of the nature of the trace quantities of carbonyl oxygen in fresh lipids has indicated that animal fats (cow's milk fat, beef fat, lard) contain 5–15  $\mu$ moles/g of predominantly saturated keto acid moieties esterified in the glycerides. Vegetable fats from freshly pressed seeds (flax, soybean, cotton, corn) contain 1–5  $\mu$ moles/g of unsaturated keto acids. Detailed study of milk fat has revealed that the major keto acid fraction is ketostearate. Lesser amounts of saturated  $C_{10}$ – $C_{16}$  keto acids and unsaturated  $C_{18}$  keto acids have also been indicated in milk fat by gas chromatography of methyl esters prepared from keto acid concentrates. Of particular interest is the composition of the milk-fat ketostearate fraction as revealed by oximolysis, Beckmann transformation, and amide hydrolysis followed by determination of the dicarboxylic acids and amines.

TABLE I  
COMPOSITION OF THE KETOSTEARIC ACID FRACTION OF MILK FAT

Acid	Mole %	
	By dicarboxylic acid analysis	By amine analysis
8-Ketostearic	1.4	1.0
9-Ketostearic	39.7	39.0
10-Ketostearic	27.0	28.0
11-Ketostearic	10.2	10.0
12-Ketostearic	4.1	5.0
13-Ketostearic	17.6	17.0

JAMES AND WEBB<sup>1</sup> have reported that milk fat contains significant quantities of 11-octadecenoic acid and 13-octadecenoic acid in addition to the expected 9-octadecenoic acid. Comparison of this pattern of unsaturation with the ketostearate composition in Table I is of speculative interest. As summarized in the review by BLOCH *et al.*<sup>2</sup>, oxygenated fatty acids seem to be involved in the aerobic conversion of saturated acids to unsaturated acids. Hydroxy acids have recently been eliminated as likely intermediates<sup>2,3</sup>. It now appears that consideration should be given to the probable occurrence of ketone structures in the bioconversion of saturated to unsaturated fatty acids.

The requirement for molecular oxygen<sup>4</sup> in the aerobic conversion of stearate to oleate is consistent with the formation of a ketone structure. By analogy to the occurrence of phosphoenolpyruvate in intermediary metabolism one might visualize the formation of a phosphate ester of the enol form of a long-chain keto acid. Cleavage of the phosphate ester at the carbon–oxygen bond in the presence of reduced pyridine nucleotide might yield an ethylenic bond and free phosphate. FLAVIN AND SLAUGHTER<sup>5</sup> have reported that a *Neurospora* enzyme cleaves *O*-phosphohomoserine to vinylglycine and phosphate.

The keto acids were isolated by reacting lipids with 2,4-dinitrophenylhydrazine

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in dry 1 N HCl-methanol. The hydrazones were isolated from the bulk of the methyl esters by chromatography on magnesia<sup>8</sup>. The hydrazones of the keto acid methyl esters were then separated from other classes of hydrazones by chromatography on grade-III (ref. 7) alumina. These keto acid derivatives were then fractionated by hexane-acetonitrile partition chromatography<sup>8</sup>. The methyl keto esters were re-generated from the hydrazones by reaction with an excess of acidic acetone. The isolated ketostearate esters from milk fat were identified by gas chromatography on Apiezon L, infrared examination, saponification equivalent, carbonyl-oxygen content, and products of the Beckmann transformation<sup>9</sup>. The dicarboxylic acids from hydrolysis of the Beckmann amides were determined by gas chromatography of their dimethyl esters on Apiezon L. The amines were determined by spectrophotometric measurement of their 2,4-dinitrophenyl derivatives after separation of the derivatives on a hexane-acetonitrile column<sup>8</sup>. Keto acids were also concentrated from fat without hydrazone formation by chromatography of both native fat and methyl ester preparations on grade-III alumina. The ketoglycerides and methyl keto esters were adsorbed from hexane solutions and eluted in a high degree of purity with methylene chloride. Detailed reports of this work will be presented.

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